Supporting Information

Monodisperse phase transfer and surface bioengineering of metal nanoparticles via silk fibroin protein corona

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Experimental Section

**Chemicals:** Silkworm cocoons were purchased from Tianyou Silk Co. Ltd. (GuangXi, China). The high-quality IONPs were purchased from Ocean NanoTech (Springdale, AR, USA). Curcumin (CUR), yttrium acetate hydrate (Y(CF₃COO)₃, 99.9%), ytterbium acetate hydrate (Yb(CF₃COO)₃, 99.9%), erbium acetate hydrate (Er(CF₃COO)₃, 99.9%), ammonium fluoride (NH₄F), 1-octadecene, oleic acid (OA), and cyclohexane were purchased from Sigma-Aldrich (St. Louis, MO, America). All other chemicals were of analytical grade and were used as received from manufacturer. All solutions were prepared with ultrapure water obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA) with resistivity of 18.2 MΩ·cm.

**Apparatus:** Transmission electron microscopy (TEM) images were carried out using a Tecnai G220 instrument (G220, FEI, Portland, America). The samples were deposited on carbon-coated 100 mesh copper grids for TEM observation at 200 kV. Dynamic Light Scattering (DLS) measurement was performed with a Zetasizer (Nano ZS, Malvern, Worcestershire, England) equipped with a 633 nm He-Ne laser using an angle of 173°. Ultraviolet-visible-near-infrared light (UV-Vis-NIR) absorption spectrum was recorded with a SH-1000 Lab microplate reader (Corona Electric, Hitachinaka, Japan). T₂-Weighted phantom images and relaxation times were measured on a 0.5 T MRI scanner (NMI20-015V, Niumag Corporation, Shanghai, China). A NIR laser (Shaanxi Kai Site Electronic Technology Co. Ltd, 980 nm, 5 W, China) was used as the excitation source for UCNPs. The fluorescence images of cells were taken on laser-scanning confocal microscopy system (Olympus, Fluoview FV1200, America). The structure of the different scaffolds was analyzed by Fourier transform infrared spectroscopy (FTIR) on a Nicolet FTIR 5700 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

**Preparation of Amorphous Silk Fibroin Aqueous Solutions:** Silk solution was prepared according to previously described methods. *Bombyx mori* cocoons were boiled for 20 min in an aqueous solution of 0.02 M Na₂CO₃, and then rinsed thoroughly with distilled water to extract the sericin proteins. The extracted silk was dissolved in 9.3 M LiBr solution (Sigma-Aldrich, St. Louis, MO, America) at 60 °C, yielding a 20% (w/v) solution. This solution was dialyzed against distilled water, using Slide-a-Lyzer dialysis cassettes (Pierce, MWCO 3500) for 72 hours to remove the salt. The solution was optically clear after dialysis and was centrifuged at 9000 rpm for 20 min at 4 °C to remove silk aggregates. The final concentration of aqueous silk solution was about 6 wt%, determined by weighing the remaining solid after drying. Fourier self-deconvolution (FSD) of the infrared spectra covering the amide I region.
(1595-1705 cm\(^{-1}\)) was performed by Peakfit4.12 software to identify silk secondary structures. FSD spectra were curve-fitted to measure the relative areas of the amide I region components.

**Preparation of β-sheet-rich Silk Fibroin Aqueous Solutions:** To prepare β-sheet-rich silk fibroin solutions, the fresh silk solutions were treated by a slow concentration-dilution process. The solution (6 wt%) was slowly concentrated to about 20 wt% over 24 hours at 60 °C to form metastable nanoparticles, and then diluted to 0.1 wt% with distilled water. The diluted silk solution was incubated for about 24 hours at 60 °C to induce the formation of β-sheet-rich silk fibroin solution. Fourier self-deconvolution (FSD) of the infrared spectra covering the amide I region (1595-1705 cm\(^{-1}\)) was performed by Peakfit4.12 software to identify silk secondary structures. FSD spectra were curve-fitted to measure the relative areas of the amide I region components.

**Synthesis of Silk Fibroin-functionalized IONPs:** The oleic acid capped IONPs in 0.2 mL of chloroform was slowly added into 0.2 mL 0.1 wt% β-sheet-rich silk fibroin solution, and the solution was vigorously stirred for 12 hours. Afterward, the IONPs were transferred into the upper water layer from the chloroform layer. Then the upper water solution was transferred to a microtube. After brief ultrasonic processing, the excess silk fibroin were removed from SF-IONPs by centrifugation and washing. Finally, the SF-IONPs were re-dispersed in water and stored at 4 °C for further use.

**Interactions of IONPs with serum proteins:** SF-IONPs and commercially available IONPs aqueous solution were respectively incubated with 10% fetal bovine serum (FBS) solutions at 37 °C for 1 hour, keeping the final iron oxide concentration constant and equal to 1 mg/ml. The samples were separated from the supernatant by centrifugation at 20,000 g for 5 minutes. The pellet was then resuspended in PBS and centrifuged again for 5 minutes at 20,000 g to pellet the particle-serum protein complexes. For SDS-PAGE, the pellet was resuspended in protein loading buffer. It was then boiled for 5 minutes at 100 °C and an equal sample volume was loaded in 10% polyacrylamide gel. The electrophoresis was performed at 120 V for 1.5 hour, until the proteins neared the end of the gel. The gels were stained for 30 minutes in Coomassie blue staining solution and destained overnight. Gels were scanned using a Biorad GS-800 calibrated densitometer scanner.

**In vitro Magnetic Resonance Imaging:** A series of SF-IONPs aqueous solutions containing different iron concentrations (0, 0.025, 0.05, 0.1, 0.2, and 0.4 mM) were prepared. \(T_2\)-weighted phantom images and relaxation times were measured on a 0.5 T MRI scanner under the following parameters: TR/TE = 2000/60 ms, 256 × 256 matrices, thickness = 1 mm.

**Magnetic Resonance Imaging of Cells:** MCF-7 cells seeded in 6-well plates (5×10\(^5\))
cells/well) were incubated with SF-IONPs at different iron concentrations (0, 0.5, 1, and 2 mM) for 2 hours, respectively. The cells were then washed with PBS and harvested to proceed MRI measurement.

**CUR Loading and Release Experiments:** To load CUR onto SF-IONPs, SF-IONPs aqueous solution was mixed with CUR in ethanol at initial concentration of 500 μg/mL iron oxide and 100 μg/mL CUR. The mixture was continuously shaken for 24 hours at 25 °C. The SF-IONP-CUR was collected by magnetic separation and washed three times with water. The loading capacity was defined as the percentage of the actual mass of CUR loaded on SF-IONPs relative to the mass of the SF-IONPs nanoplates. The equation is provided followed. The amount of drug-loaded CUR was determined through UV-Vis-NIR absorption spectrum at 425 nm.

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\text{Loading capacity (\%)} = \frac{W_{\text{initial CUR}} - W_{\text{CUR in supernatant}}}{W_{\text{nanomaterials}}} \times 100\% 
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In order to study the release kinetics of CUR, the SF-IONP-CUR complex dispersed in 0.2 mL of PBS buffer in dialysis membrane tubing was equilibrated with 3 mL of PBS buffer supplemented with 0.1% Tween 80, and then 100 μL aliquots were removed for absorbance spectrometer measurements. After each measurement, the each 100 μL aliquot was returned to the original solution for maintaining total volume and total CUR amount.

**Cell Culture and Cytotoxicity Assay:** MCF-7 cells were seeded in 96-well plates and then incubated with different concentrations of SF-IONPs, free CUR and SF-IONP-CUR for 24 hours at 37 °C in a humidified 5% CO₂ atmosphere. The standard MTT assay was carried out to determine the cell viabilities to the control untreated cells.

**Synthesis of NaYF₄:Er/Yb@NaYbF₄@NaYF₄ UCNPs:** Multilayered core-shell nanoparticles were synthesized according to a literature procedure. Y(CF₃COO)₃, Yb(CF₃COO)₃, Er(CF₃COO)₃, oleic acid and 1-octadecene (ODE) were firstly used to prepare Er/Yb codoped NaYF₄ core nanoparticles. Then, the presynthesized NaYF₄:Er/Yb core nanoparticles were used as seeds for shell modification. A cyclohexane dispersion of NaYF₄:Er/Yb nanoparticles was reacted with Yb(CF₃COO)₃, NaOH and NH₄F to form the core-shell NaYF₄:Er/Yb@NaYbF₄ nanoparticles. Finally, the NaYF₄:Er/Yb@NaYbF₄ core-shell nanoparticles were used as seeds and conformally coated with a thin layer of NaYF₄ to synthesize the multilayered core-shell nanoparticles.

**Synthesis of Silk Fibroin-functionalized UCNPs:** The oleic acid capped UCNPs in 0.2 mL of cyclohexane was slowly added into 0.2 mL 0.1 wt% β-sheet-rich silk fibroin solution, and the solution was vigorously stirred for 12 hours. Afterward, the UCNPs were transferred into the
upper water layer from the cyclohexane layer. Then the upper water solution was transferred to a microtube. After brief ultrasonic processing, the excess silk fibroin were removed from SF-UCNPs by centrifugation and washing. Finally, the SF-UCNPs were re-dispersed in water and stored at 4 °C for further use.

**Cancer Cells Fluorescence Imaging:** MCF-7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) media with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 mg/mL) at 37 °C in a 5% CO₂ incubator. The cells were plated at 60% - 70% confluency for 24 hours before imaging experiments in 35 mm culture dishes. Cultured cells were washed with PBS (pH 7.4) two times prior to imaging. After incubation with SF-UCNPs for 4 hours, medium was removed. The treated MCF-7 cells were washed with PBS (pH 7.4). Thereafter, fluorescence images were immediately taken using laser-scanning confocal microscopy system (Olympus, Fluoview FV1200, America), modified to allow illumination with a NIR 980 nm laser.
Fig. S1. The second derivative Fourier transform infrared spectra of amorphous silk fibroin and β-sheet-rich silk fibroin.

Fig. S2. (a) TEM image of IONPs before β-sheet-rich silk fibroin modification in chloroform. (b) TEM image of IONPs after β-sheet-rich silk fibroin modification in water.
Fig. S3. (a) Dynamic light scattering (DLS) data of unmodified IONPs in chloroform (black) and SF-IONPs in water (red). (b) UV-Vis spectra of unmodified IONPs in chloroform (black) and SF-IONPs in water (red). A characteristic peak of protein at 280 nm after silk fibroin modification suggested the successful attachment of protein to IONPs.

Fig. S4. (a) The second derivative Fourier transform infrared spectra of β-sheet-rich silk fibroin and SF-IONPs. A characteristic peak of β-sheet conformation at 1627 cm$^{-1}$ suggested the successful attachment of protein to IONPs. (b) SDS-PAGE and Coomassie Brilliant Blue staining of unmodified IONPs and SF-IONPs. A characteristic peak of silk fibroin below 25 kDa suggested the successful attachment of protein to IONPs.
Fig. S5. TGA curves of SF-IONPs prepared by changing initial volume ratio between hydrophobic IONPs and 0.1 wt% β-sheet-rich silk fibroin (IONPs/SF) from 2:1, 1:1 to 1:2 during the phase-transfer process.

Fig. S6. (a) TEM images of IONPs in water after phase-transfer by silk fibroin with the amorphous conformation. (b) TEM images of IONPs in water after phase-transfer by bovine serum albumin (BSA).
Fig. S7. Dynamic light scattering (DLS) data of IONPs in water after phase-transfer by silk fibroin with the amorphous conformation (red) and bovine serum albumin (black).

Fig. S8. Photos of SF-IONPs in different solutions including PBS, FBS and RPMI-1640 with 10% fetal bovine serum (FBS) cell culture medium after one month at 4 °C.
Fig. S9. Dynamic light scattering (DLS) data of SF-IONPs in different solutions including PBS, FBS and RPMI-1640 with 10% fetal bovine serum (FBS) cell culture medium after one month at 4 °C.

Fig. S10. The SF-IONPs in the aqueous solutions with different pH values after 48 hours.

Fig. S11. (a) Fluorescence spectra of free CUR (black) and SF-IONP-CUR (red). (b) CUR release profile of SF-IONP-CUR.
Fig. S12. (a) TEM images of UCNPs before β-sheet-rich silk fibroin modification in cyclohexane. (b) TEM images of UCNPs after β-sheet-rich silk fibroin modification in water. (c) Photograph of solvent dispersity of UCNPs (left) before and (right) after β-sheet-rich silk fibroin modification under ambient light.

Fig. S13. (a) Dynamic light scattering (DLS) data of unmodified UCNPs in cyclohexane (black) and SF-UCNPs in water (red). (b) UV-Vis spectra of unmodified UCNPs in cyclohexane (black) and SF-UCNPs in water (red). (c) Room-temperature up-conversion
luminescence spectrum of UCNPs in cyclohexane (black) and SF-UCNPs in water (red) under excitation at 980 nm.

Fig. S14. Photos of SF-UCNPs in different solutions including PBS, FBS and RPMI-1640 with 10% fetal bovine serum (FBS) cell culture medium after one month at 4 °C. For each picture, photographs of SF-UCNPs were taken under ambient light (left) and corresponding luminescence photograph under excitation by a 980 nm laser (right).

Fig. S15. Dynamic light scattering (DLS) data of SF-UCNPs in different solutions including PBS, FBS and RPMI-1640 with 10% fetal bovine serum (FBS) cell culture medium after one month at 4 °C.
Fig. S16. Confocal microscopy images of MCF-7 cells treated with SF-UCNPs. Each series can be classified as the bright-field (left) and overlay of both bright-field and upconversion luminescent images (right), respectively.